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Translation-125-1 Against Blanket Agreement No. FD 3-5839 (Italian-English) Center of Applied Military Nuclear Energy - (C.A.M.E.N.), S. Piero a Grado (Pisa). Laboratory of Radiopathology and Hygiene of Radiations. (Director: Professor Arghittu)

STUDIES AND EXPERIMENTS OF RADIOMICROBIOLOGY

III. Topographic Distribution of the Staphylococcal Enterotoxin Labelled I^{131} in Responsive Animals (young cats)

(C. Arghittu, L. Lenzerini and M. Rossi-Torelli)

The staphylococcal enterotoxin (E.S.) has recently been purified by Bergdol, Sugiyama and Dack (2). Its molecular weight is 24,000 and its chemical composition includes seventeen amino-acids the most abundant of which are aspartic acid, lysin and thyroxin.

Pure staphylococcal enterotoxin is endowed with antigenic properties and therefore yields, with the corresponding anti-serum, a series of precipitates which can be made discernable by means of the technique of Oudin and Ouchterlony.

The points of attack of staphylococcal enterotoxin on sensitive animals are poorly known. The mechanism of action of the enterotoxin in provoking the gastroenteric syndrome has not yet been clarified sufficiently to say whether it is in the form of a stimulus of the central nervous system, or a peripheric stimulus at the level of the gastric and intestinal mucous.

As a result, it has appeared useful to start a series of tests and experiments using an enterotoxin marked with various radioisotopes, in an attempt to contribute to the study of this problem, which is still unsolved. In this first experiment we refer to the results achieved when using enterotoxin marked with I^{131}.

MATERIALS AND METHODS

<u>Enterotoxic staphylococci stock</u>

We have used in our experiment stock No. 243, which had been kindly provided by Dr. Casman, of the Food & Drug Administration in Washington, D. C. That germ had been isolated from a hotbed of enteritis and had appeared particularly toxigenous in many biological toxigenity tests which we carried out on young cats.

Culture filtrates of stock 243, treated at 100° during 30 minutes, and inoculated intraperitoneally, have constantly caused vomiting and

diarrhea followed by a state of depression among a high percentage of the inoculated kittens.

Culture medium and production of enterotoxin

We have used a liquid ground constituted as follows: Bacto Casamino Acids (Difco) 15 gr.; Nicotinic acid 0.00123 gr.; Thiamine 0.00005 gr.; and glucose 2.25 gr. per liter. The liquid ground was poured into 10 liter Pyrex glass vats which had been sterilized in autoclave during 30 minutes. The seeding of the vats with fresh culture of 12 hours developed in broth made from heart and brains. Incubation in thermostat at 37^{o} per 5 gg. with continuous agitation of the cultures with pallets, and with a continuous flow of a gas mixture made up of 90% oxygen and 10% carbon anhydride. Centrifugation and filtration of cultures by Seitz. Treatment of the floating part at 100^{o} during 30 minutes.

Purification of enterotoxin

We have followed the method described by Bergdol and his colleagues (2), modifying it the first time as follows:

- 20 liters of bacterial filtrate (the germs have been removed by filtering through Seitz) have been treated for 30 minutes at 100^{o}. We have then added to it, drop by drop, H_2SO_4 1N until a 10% pH Natrium Metaphosphate of 3.3, controlling meanwhile the temperature of the mixture so that it should not surpass 1^{o}. At the end of this operation, we added for each liter of mixture 5 gr. of Hyflo super cell, whereupon the suspension was submitted to stirring during 1 hour at 0^{o}. The suspension was collected by filtration over a layer of Hyflo super cell (1 gr./liter) at a temperature of 4^{o}. The precipitate has been extracted in three separate extractions with a total of 5 cc of Na_2HPO_4 0.0M per each gram of Hyflo used.

In the second and third time the method of Bergdol was followed closely. After precipitation with ethanol it has not been possible ultimately to purify staphylococcal enterotoxin because the amount of the substance which had been obtained was too small.

Table 2
Response of the Cats to the Administration of Partially Purified Enterotoxin Preparations

Sample	Gamma cc proteins	Number of treated animals	Amount Adm. Endoperitoneally	Response (vomiting, diarrhea)
Bacterial filtrate for 30' at 100° C	990	4	5 cc	100%

Table 2 continued

Sample	Gamma cc proteins	Number of treated animals	Amount Adm. Endoperitoneally	Response (vomiting, diarrhea)
Eluted Hyflo Super Cell dialized against distilled H_2O	471	4	4 cc	100%
Eluted alumina column dialized against distilled H_2O	37	4	4 cc	100%
Precipitate with alcohol dialized against distilled H_2O	9.25	4	4 cc	100%

Marking enterotoxin with I^{131}

The lyphilated sample of enterotoxin has been brought back in suspension with a stopped solution of sodium phosphate and has been marked with I^{131}, according to the technique described by Gilmore and colleagues (3). The amount of I^{131} employed was 1 mc. After the last period of the process and after the dialises, the radioactivity of the sample has been measured by means of a perforated scintillometer and was found to be 1,800,000 c/m per cc. It is interesting to stress the fact that the process of marking enterotoxin with I^{131} has not altered the specific property of the toxin, inasmuch as all the cats inoculated with marked enterotoxin have shown within two hours of the injection repeated vomiting and diarrhea.

Animals Used

10 young cats were used, whose average weight was 1531 grams and who were divided into five groups of two animals. The small number of animals in each group was imperative because of the notable difficulty of finding simultaneously a substantial number of young cats. In each group one cat was injected intraperitoneally with 4.8 cc of marked enterotoxin containing a radioactivity of 8,640,000 c/m, while the other cat, who served as control animal, was injected with [illegible]lution of I^{131} containing equal activity. The groups were put to death 1/2 hour, 1, 2, 4, 24 hours after the inoculation. The following extractions were effected from each cat: blood, encephalon, stomach, thin intestine, liver, spleen, suprarenal capsules, kidneys. The radioactivity of samples of single organs was determined in a perforated scintillator connected with an analyser of impulses. In order to be able to study better the distribution of enterotoxin in the various sections of the

central nervous system, the following determinations were constantly followed there: one in the telecephalon, one in the mesencephalon, one in the bridge and bulb and one in the cerebellum. The radioactivity in the single organs and tissues, expressed in shots gr/min. and in percentage of the injected dose is given in the following table.

Table 1

Radioactivity of Various Organs of Animals Treated with Marked Toxin and of Animals Serving as Controls Sacrificed at Various Intervals After the Inoculation

1st group (1/2 hours after inoculation)

	Treated with Enterotoxin		Control Animals	
	cm/gr	% of injected dose	cm/gr	% of injected dose
Blood	4307	0.042	21791	0.21
Telencephalon	245	0.0024	2435	0.0239
Mesencephalon	229	0.0022	835	0.0022
Bridge and bulb	249	0.0024	1405	0.0138
Cerebellum	224	0.0022	1622	0.016
Thyroid	1910	0.018	7069	0.069
Lungs	1732	0.017	15047	0.148
Heart	1542	0.015	8669	0.08
Stomach	11165	0.11	48358	0.476
Thin intestine	3980	0.039	13757	0.135
Liver	6130	0.06	13624	0.134
Spleen	9110	0.089	12670	0.125
Kidneys	13256	0.13	14641	0.144
Suprarenals	34578	0.34	13504	0.133
Urine	10576	0.1	1914	0.0188

(Groups II, III, IV, V with parts of body in the same order)
SEE ATTACHED SHEETS FOR TABLES II, II, IV & V AND GRAPHS

RESULTS AND DISCUSSION

Observing the data shown on the table and the movement of radioactivity in function of time for any single organ or tissue, which result from the graphs shown here, the following is noticed:

1. The radioactivity of the controls, at the various periods of the experiment, is notably higher than the radioactivity of the cats treated with enterotoxin, in almost all the organs and tissues which were considered, except in the kidneys and in the suprarenal capsules. This higher radioactivity of the controls must be attributed to a more rapid absorbiton and to a more rapid distribution of the radioactive Iodine, which, in this group of animals, is free in the peritoneal cavity, while in the group of animals who had been treated, that is firmly tied to the big molecule

of enterotoxin, which is absorbed slowly

That interpretation is in conformity with the course of the curve of total absorbtion, expressed in percentage of the injected dose, in the groups of animals both of those treated with enterotoxin and of those kept as controls. Graph No. 5 shows clearly that the absorbtion and the distribution in time of radioactivity, in animals of the first group, are slower and more uniform than in the case of those of the second group (controls) in whom a rapid rise of radioactivity took place and was followed by a rapid fall in radioactivity.

It seems then that finding a high level of radioactivity in the kidneys and in the suprarenal capsules of the animals treated with enterotoxin is particularly significant, inasmuch as it appears to indicate a specific localization of the enterotoxin in those two organs, which would thus take the role of targets or points of attack of the venom. The hypotension, the dejection and the prostration which always accompany the other symptoms which characterize the gastro-enteric syndrome of enterotoxin, could be the expression of an acute insufficiency of suprarenal capsules caused by the localization and by the attack of the toxin.

2. Radioactivity at the level of gastric mucous and the mucous of the thin intestine, is very high both in the controls and in the treated animals. That phenomenon is explained with the normal metabolic comportment of the Iodine, which is normally eliminated through the gastroenteric mucous membrane.

3. The radioactivity of the encephalic mass is scarce and uniformly distributed in the two groups of animals. It seems possible to deduce from this observation that there are no special encephalic zones and centers where the enterotoxin would be localized and where it would exercise its specific action (unless one does not wish to suppose that enterotoxin would act in minima doses on certain recipients). The emetic effect, which is the main and the most characteristic of the effects of enterotoxin, would then have to be attributed to an action of peripheric stimulation at the level of gastrointestinal mucous membrane rather than to a central stimulation action at the level of the encephalitic centers of vomiting.

The results we have expounded are not final. They need ulterior controls and repeated conformity. It is our intention, therefore, to continue our experiments, repeating the study of the distribution of enterotoxin marked with other radioisotopes.

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SEE PAGE 4 [illegible] same order as Table 1

(segue tabella N° 1)

	II° Gruppo (1 h dall'inoculazione)			
	Tratt. con enterotossina		Controlli	
	cm/gr	% dose iniettata	cm/gr	% dose iniettata
Sangue	3900	0,036	21750	0,217
Telencefalo	636	0,006	1267	0,012
Mesencefalo	476	0,0048	1006	0,0099
Ponte e bulbo	1630	0,016	1572	0,015
Cervelletto	426	0,004	1562	0,015
Tiroide	3228	0,032	7283	0,072
Polmoni	3626	0,038	15938	0,157
Cuore	1536	0,015	10579	0,104
Stomaco	9996	0,098	109252	1,076
Tenue	3267	0,032	12706	0,126
Fegato	13384	0,131	11533	0,114
Milza	2692	0,026	15731	0,155
Reni	18165	0,179	15866	0,155
Surrenali	9061	0,089	12108	0,119
Urine	14776	0,14	7032	0,0620

(segue tabella N° 1)

	III° Gruppo (2 h dall'inoculazione)			
	Tratt. con enterotossina		Controlli	
	cm/gr	% dose iniettata	cm/gr	% dose iniettata
Sangue	3121	0,0308	12026	0,118
Telencefalo	1319	0,013	3233	0,0318
Mesencefalo	564	0,0055	1081	0,0106
Ponte e bulbo	677	0,0067	2179	0,0215
Cervelletto	1667	0,0164	737	0,0072
Tiroide	5455	0,0537	6450	0,0635
Polmoni	5681	0,056	8214	0,081
Cuore	2579	0,0254	5676	0,056
Stomaco	12296	0,121	64281	0,633
Tenue	4351	0,043	7218	0,0711
Fegato	4501	0,0443	3916	0,0383
Milza	5034	0,0496	7732	0,0763
Reni	25632	0,252	7071	0,0778
Surrenali	7034	0,0697	5729	0,0564
Urine			34037	0,340

SEE PAGE 4 parts of body same as in table 1

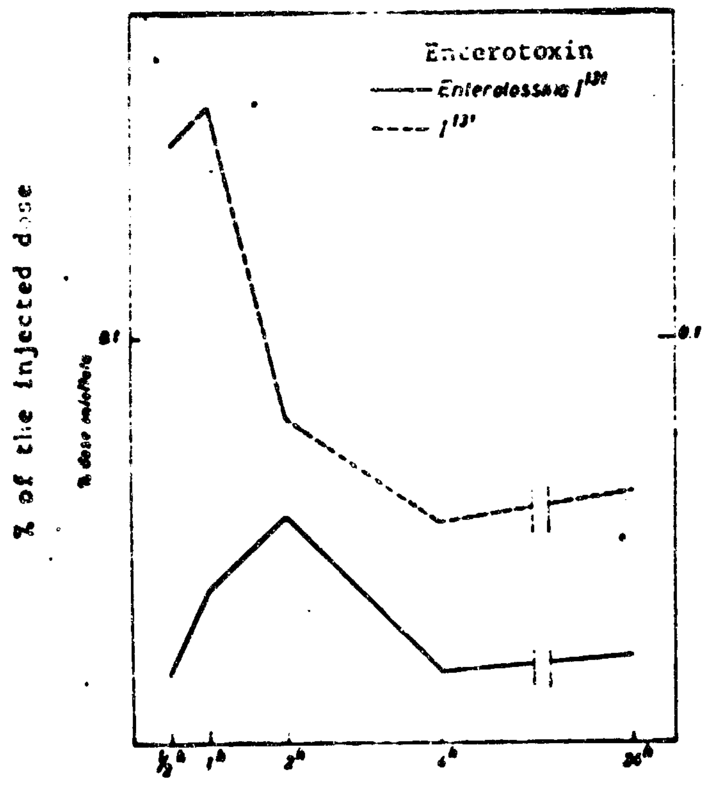
(segue tabella N° 1)

	IV° Gruppo (4 h dall'inoculazione)			
	Tratt. con enterotossina		Controlli	
	cm/gr	% dose iniettata	cm/gr	% dose iniettata
Sangue	[illegible]33	0,027	8244	0,081
Telencefalo	613	0,006	616	0,006
Mesencefalo	591	0,0056	658	0,0065
Ponte e bulbo	664	0,0063	668	0,0065
Cervelletto	349	0,0037	585	0,0057
Tiroide	1472	0,014	4137	0,040
Polmoni	1920	0,019	5592	0,055
Cuore	2454	0,024	4633	0,045
Stomaco	12916	0,127	25940	0,240
Tenue	4678	0,046	5024	0,049
Fegato	4287	0,042	3946	0,0388
Milza	4407	0,043	4108	0,040
Reni	20458	0,201	5641	0,055
Surrenali	5213	0,051	2768	0,027
Urine	93395	0,920	37782	0,371

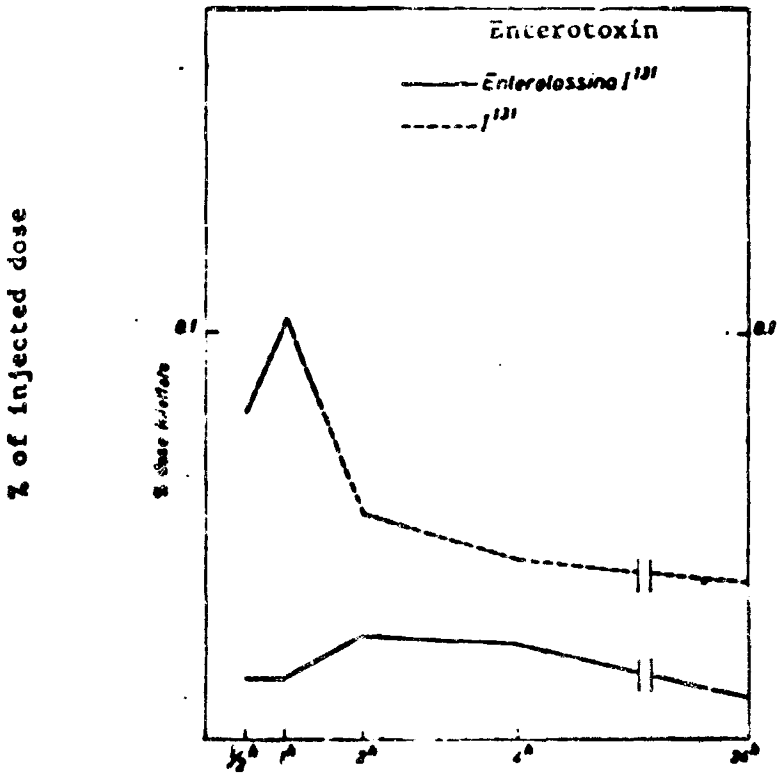
(segue tabella N° 1)

	V° Gruppo (24 h dall'inoculazione)			
	Tratt. con enterotossina		Controlli	
	cm/gr	% dose iniettata	cm/gr	% dose iniettata
Sangue	2542	0,0250	9147	0,0901
Telencefalo	171	0,0016	396	0,0039
Mesencefalo	186	0,00183	446	0,0044
Ponte e bulbo	211	0,00207	470	0,0046
Cervelletto	174	0,00171	359	0,0035
Tiroide	1932	0,0190	3867	0,0381
Polmoni	2283	0,0225	6461	0,0636
Cuore	1042	0,0168	3961	0,0390
Stomaco	3257	0,0322	21806	0,2071
Tenue	1285	0,0127	4258	0,0419
Fegato	[illegible]	0,0491	4237	0,0417
Milza	2337	0,0230	4852	0,0470
Reni	37432	0,3658	5662	0,0558
Surrenali	1006	0,0099	3804	0,0063
Urine	25233	0,2486	49796	0,4906

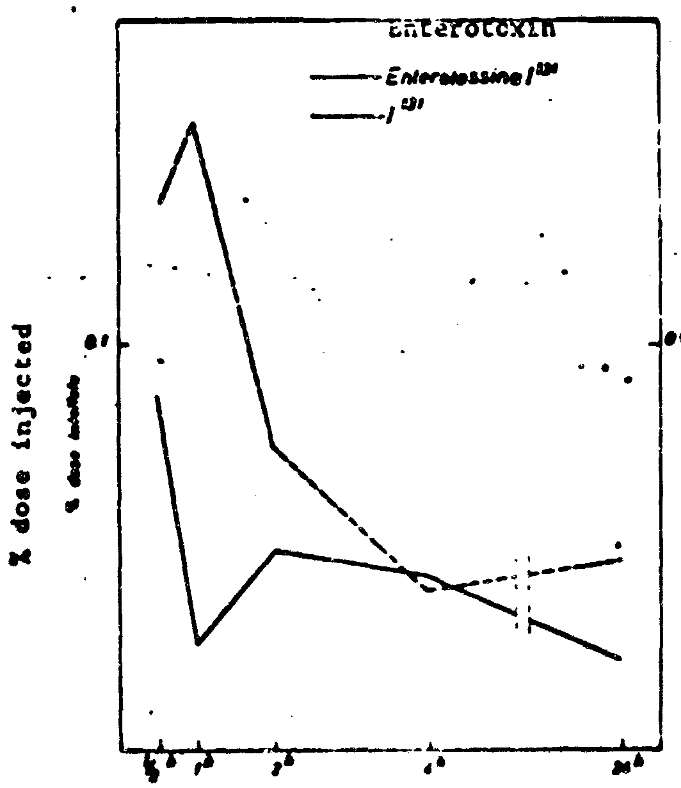
Graph No. 1: Comportmen[illegible]
in the blood of young [illegible]
marked I^{131} and of a [illegible]



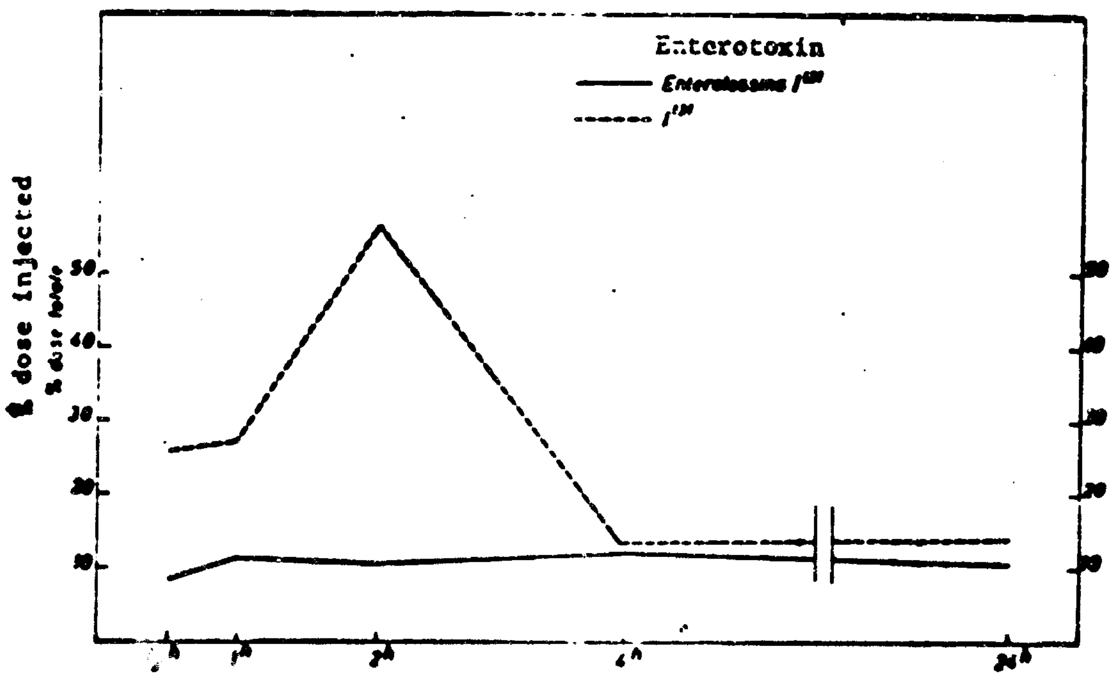
Graph No. 2
Comportment of the radioactivity in function of time in the lungs of young cats after administration of enterotoxin marked I^{131} and of solution of I^{131}.



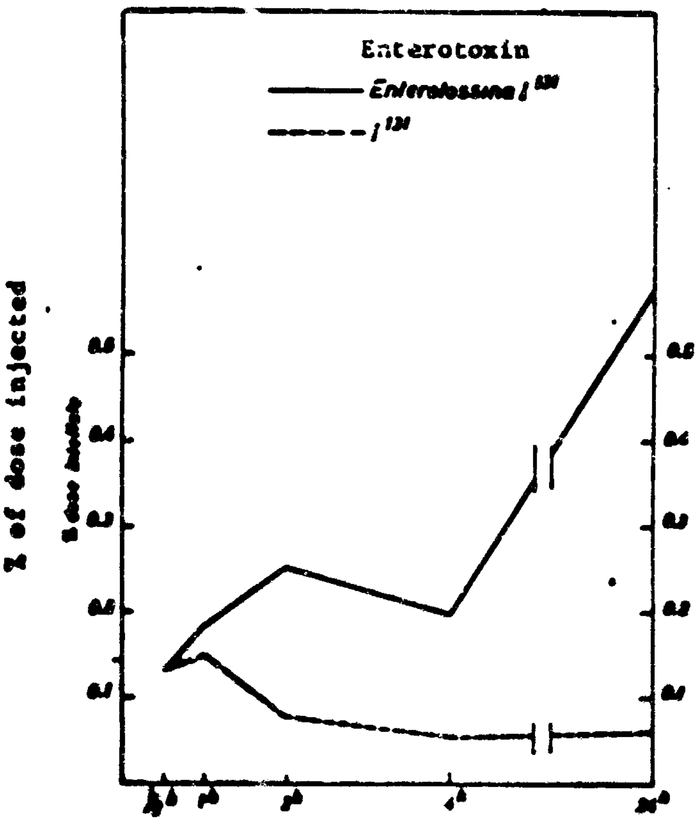
Graph No. 3 Comportment of radioactivity in function of time in the heart of young cat- after administration of enterotoxin marked I^{131} and of solution of I^{131}.



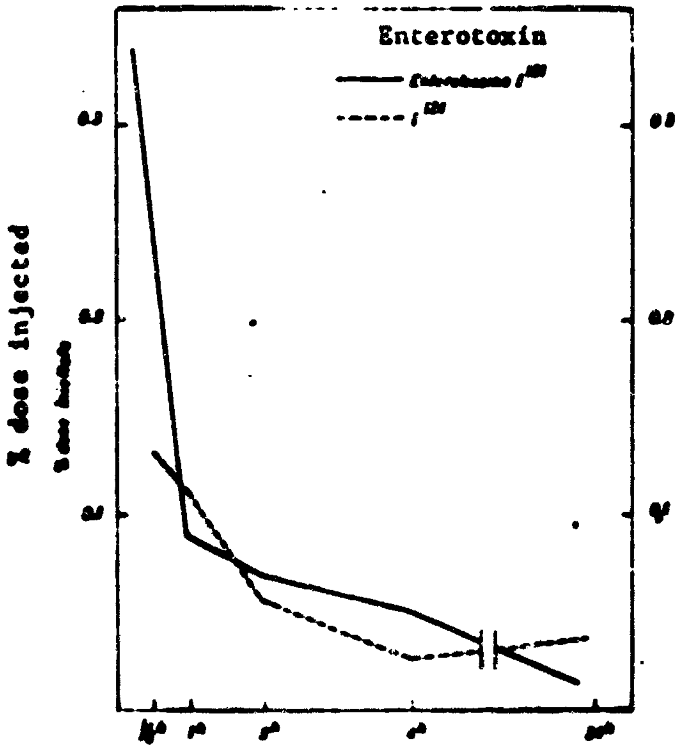
GraphNo. 4 comportment of radioactivity in function of time in the spleen of young cats after administration of enterotoxin marked I^{131} and of solution of I^{131}.



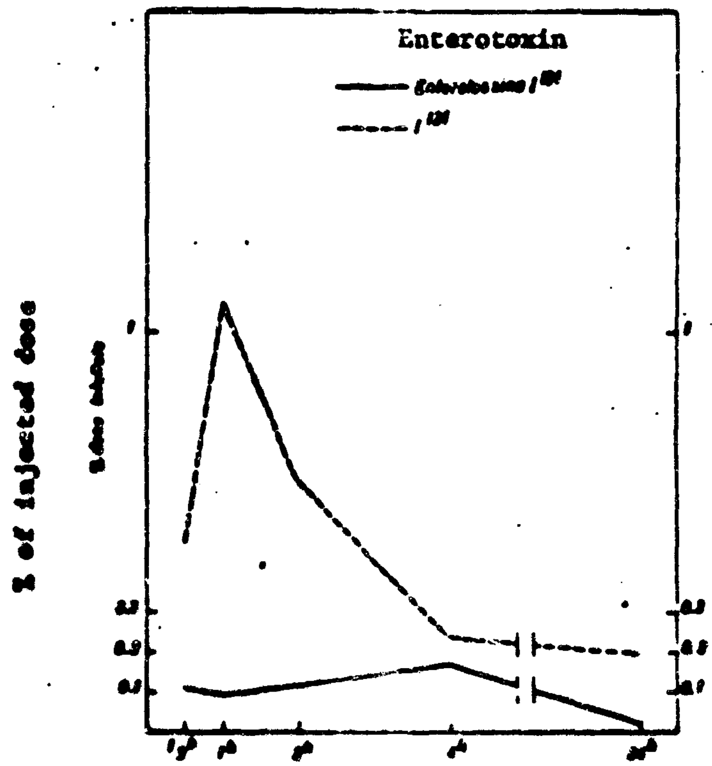
Graph No. 5. Comportment of global radioactivity in cats treated with Enterotoxin I^{131} and with solution of I^{131}.



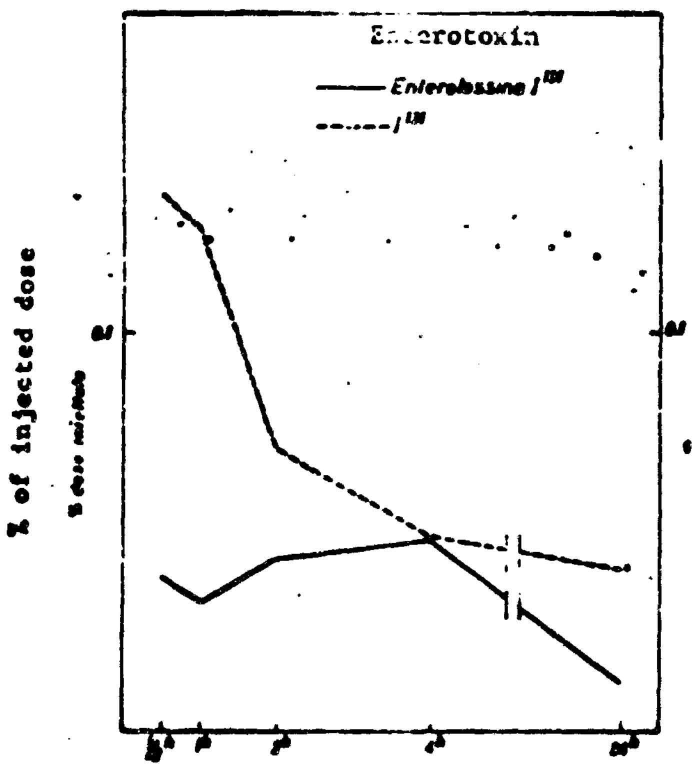
Graph No. 6 Compartment of radioactivity **in function of time in the kidneys of young** cats after **administration of enterotoxin marked** I^{131} **and** of solution of I^{131}.



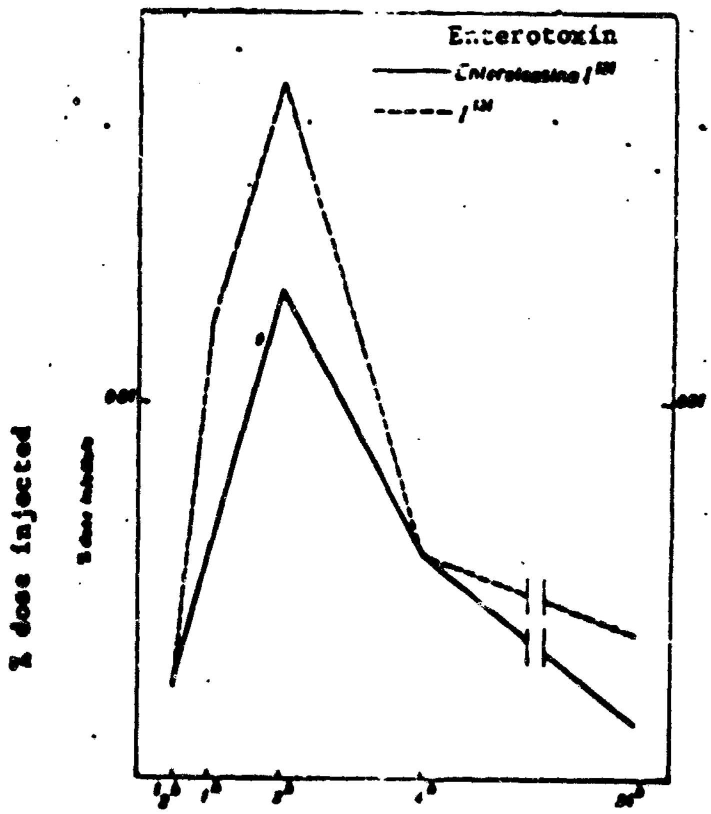
Graph No. 7 Compartment of radioactivity in function of time in the suprarenal capsules of young cats after administration of enterotoxin marked I^{131} and of solution of I^{131}.



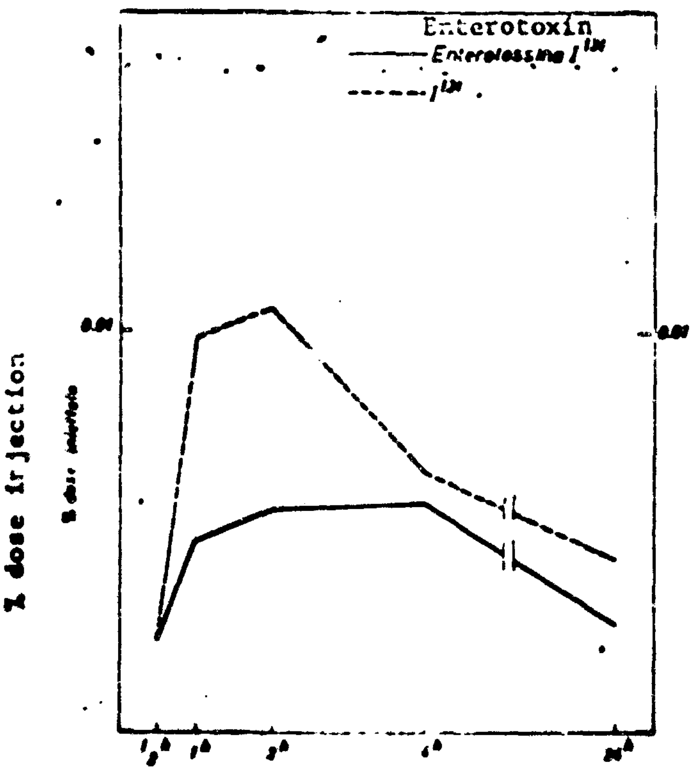
Graph No. 8: Compartment of Radioactivity in function of time in the stomach of young cats after administration of enterotoxin marked I^{131} and of solution of I^{131}.



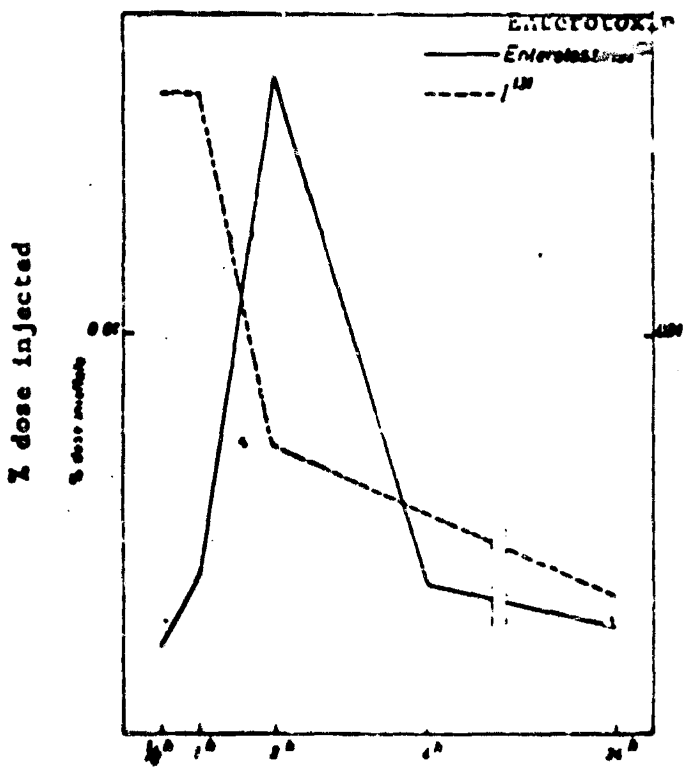
Graph No. 9: Comportment of radioactivity in function of time in the thin gut of young cats after administration of enterotoxin marked I^{131} and of solution of I^{131}.



Graph No. 10: Comportment of radioactivity in function of time in the telecephalon of young cats after administration of enterotoxin marked I^{131} and of solution of I^{131}.



Graph No. 11 Comportment of radioactivity in function of time in the mesencephalon of young cats after administration of enterotoxin marked I^{131} and of solution of I^{131}.



Graph No. 12. Comportment of radioactivity in function of time in the cerebellum of young cats after administration of enterotoxin marked I^{131} and of solution of I^{131}.